=> d l2 abs ibib kwic 26 29

L2 ANSWER 26 OF 33 HCAPLUS COPYRIGHT 2004 ACS on STN

AB A method for **stabilizing** intracellular proteins known to cause disease, said method comprising contacting a cell with **stabilizing** agents such as DMSO, sugars, amino acids and TMAO (trimethylamine N-oxide), wherein the proteins are **stabilized** and the disease state lessened.

ACCESSION NUMBER: 1997:684267 HCAPLUS
DOCUMENT NUMBER: 127:341811
TITLE: Correction of genetic defects using chemical **chaperones**
INVENTOR(S): Welch, William J.; Brown, C. Randell; Tatzelt, Jorg
PATENT ASSIGNEE(S): Regents of the University of California, USA; Welch, William J.; Brown, C. Randell; Tatzelt, Jorg
SOURCE: PCT Int. Appl., 86 pp.
CODEN: PIXXD2
DOCUMENT TYPE: Patent
LANGUAGE: English
FAMILY ACC. NUM. COUNT: 2
PATENT INFORMATION:

| PATENT NO. | KIND | DATE | APPLICATION NO. | DATE |
|---|---|---|---|---|
| WO 9737645 | A1 | 19971016 | WO 1997-US5846 | 19970409 |
| W: AL, AM, AT, AU, AZ, BA, BB, BG, BR, BY, CA, CH, CN, CU, CZ, DE, DK, EE, ES, FI, GB, GE, GH, HU, IL, IS, JP, KE, KG, KP, KR, KZ, LC, LK, LR, LS, LT, LU, LV, MD, MG, MK, MN, MW, MX, NO, NZ, PL, PT, RO, RU, SD, SE, SG, SI, SK, TJ, TM, TR, TT, UA, UG, US, UZ, VN, YU, AM, AZ, BY, KG, KZ, MD, RU, TJ, TM | | | | |
| RW: GH, KE, LS, MW, SD, SZ, UG, AT, BE, CH, DE, DK, ES, FI, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE, BF, BJ, CF, CG, CI, CM, GA, GN, ML, MR, NE, SN, TD, TG | | | | |
| CA 2250780 | AA | 19971016 | CA 1997-2250780 | 19970409 |
| AU 9724485 | A1 | 19971029 | AU 1997-24485 | 19970409 |
| AU 734905 | B2 | 20010628 | | |
| EP 959877 | A1 | 19991201 | EP 1997-920243 | 19970409 |
| R: AT, BE, CH, DE, DK, ES, FR, GB, GR, IT, LI, LU, NL, SE, MC, PT, IE, FI | | | | |
| JP 2000509971 | T2 | 20000808 | JP 1997-536453 | 19970409 |
| US 2001021500 | A1 | 20010913 | US 2001-823657 | 20010330 |
| US 6541195 | B2 | 20030401 | | |

PRIORITY APPLN. INFO.:

| | | |
|---|---|---|
| US 1996-15155P | P | 19960410 |
| US 1997-838691 | A2 | 19970409 |
| WO 1997-US5846 | W | 19970409 |
| US 1999-291406 | A1 | 19990413 |

TI Correction of genetic defects using chemical **chaperones**

AB A method for **stabilizing** intracellular proteins known to cause disease, said method comprising contacting a cell with **stabilizing** agents such as DMSO, sugars, amino acids and TMAO (trimethylamine N-oxide), wherein the proteins are **stabilized** and the disease state lessened.

ST genetic disease **conformation** therapy **chaperone** chem

IT Brain, disease
Prion diseases
(Creutzfeldt-Jakob; correction of genetic defects using chemical **chaperones**)

IT Lipoprotein receptors

RL: ADV (Adverse effect, including toxicity); BPR (Biological process); BSU (Biological study, unclassified); PRP (Properties); BIOL (Biological study); PROC (Process)
(LDL, **mutant**; correction of genetic defects using chemical **chaperones**)

IT Prion proteins
RL: ADV (Adverse effect, including toxicity); BPR (Biological process); BSU (Biological study, unclassified); PRP (Properties); BIOL (Biological study); PROC (Process)
(PrPc, **mutant**; correction of genetic defects using chemical **chaperones**)

IT Prion proteins
RL: ADV (Adverse effect, including toxicity); BPR (Biological process); BSU (Biological study, unclassified); PRP (Properties); BIOL (Biological study); PROC (Process)
(PrPSc, **mutant**; correction of genetic defects using chemical **chaperones**)

IT Gangliosidosis
(Tay-Sachs disease; correction of genetic defects using chemical **chaperones**)

IT Nervous system
(amyotrophic lateral sclerosis; correction of genetic defects using chemical **chaperones**)

IT Liver, disease
(chronic; correction of genetic defects using chemical **chaperones**)

IT Alzheimer's disease
Antitumor agents
Cataract
Emphysema
Marfan syndrome
Neoplasm
Scurvy
**Stabilizing** agents
(correction of genetic defects using chemical **chaperones**)

IT **Chaperonins**
RL: BAC (Biological activity or effector, except adverse); BPR (Biological process); BSU (Biological study, unclassified); THU (Therapeutic use); BIOL (Biological study); PROC (Process); USES (Uses)
(correction of genetic defects using chemical **chaperones**)

IT Amino acids, biological studies
Carbohydrates, biological studies
Polyoxyalkylenes, biological studies
RL: BAC (Biological activity or effector, except adverse); BSU (Biological study, unclassified); THU (Therapeutic use); BIOL (Biological study); USES (Uses)
(correction of genetic defects using chemical **chaperones**)

IT Nervous system
(disease, Gerstmann-Straussler syndrome; correction of genetic defects using chemical **chaperones**)

IT Hypercholesterolemia
(familial; correction of genetic defects using chemical **chaperones**)

IT Brain, disease
Prion diseases
(fatal familial insomnia; correction of genetic defects using chemical **chaperones**)

IT Insomnia
(fatal familial; correction of genetic defects using chemical

**chaperones**)
IT Disease, animal
(genetic, protein **conformation** defects from; correction of genetic defects using chemical **chaperones**)
IT Endocrine system
(leprechaunism; correction of genetic defects using chemical **chaperones**)
IT Mental disorder
(maple syrup urine disease; correction of genetic defects using chemical **chaperones**)
IT CFTR (cystic fibrosis transmembrane conductance regulator)
Collagens, biological studies
Crystallins
Fibrillins
Insulin receptors
Prion proteins
Rhodopsins
**p53** (protein)
RL: ADV (Adverse effect, including toxicity); BPR (Biological process); BSU (Biological study, unclassified); PRP (Properties); BIOL (Biological study); PROC (Process)
(**mutant**; correction of genetic defects using chemical **chaperones**)
IT Bone, disease
(osteogenesis imperfecta; correction of genetic defects using chemical **chaperones**)
IT Alcohols, biological studies
RL: BAC (Biological activity or effector, except adverse); BSU (Biological study, unclassified); THU (Therapeutic use); BIOL (Biological study); USES (Uses)
(polyhydric; correction of genetic defects using chemical **chaperones**)
IT Collagens, biological studies
RL: ADV (Adverse effect, including toxicity); BPR (Biological process); BSU (Biological study, unclassified); PRP (Properties); BIOL (Biological study); PROC (Process)
(procollagens, type I, pro-$\alpha$, **mutant**; correction of genetic defects using chemical **chaperones**)
IT **Conformation**
(protein; correction of genetic defects using chemical **chaperones**)
IT Eye, disease
(retinitis pigmentosa; correction of genetic defects using chemical **chaperones**)
IT Brain, disease
Prion diseases
(scrapie; correction of genetic defects using chemical **chaperones**)
IT Brain, disease
(spongiform encephalopathy; correction of genetic defects using chemical **chaperones**)
IT Amyloid
RL: ADV (Adverse effect, including toxicity); BPR (Biological process); BSU (Biological study, unclassified); PRP (Properties); BIOL (Biological study); PROC (Process)
($\beta$-; correction of genetic defects using chemical **chaperones**)
IT Animal cell line
($\Delta$F508; correction of genetic defects using chemical

**chaperones**)
IT 9067-96-3, α-Ketoacid dehydrogenase
RL: ADV (Adverse effect, including toxicity); BPR (Biological process); BSU (Biological study, unclassified); PRP (Properties); BIOL (Biological study); PROC (Process)
(correction of genetic defects using chemical **chaperones**)
IT 56-12-2, Gaba, biological studies 56-40-6, Glycine, biological studies 56-41-7, Alanine, biological studies 56-81-5, Glycerol, biological studies 56-86-0, Glutamic acid, biological studies 67-68-5, Dmso, biological studies 87-89-8, Inositol 99-20-7D, Trehalose, isofluoroside 107-35-7, Taurine 107-43-7, Betaine 107-97-1, Sarcosine 147-85-3, Proline, biological studies 149-32-6, Erythritol 1184-78-7, Trimethylamine N-oxide 7789-20-0, Water-d2 25322-68-3, Polyethylene glycol 34522-32-2, Octopine
RL: BAC (Biological activity or effector, except adverse); BSU (Biological study, unclassified); THU (Therapeutic use); BIOL (Biological study); USES (Uses)
(correction of genetic defects using chemical **chaperones**)
IT 302-95-4, Sodium deoxycholate 9002-93-1, Triton x-100
RL: NUU (Other use, unclassified); USES (Uses)
(correction of genetic defects using chemical **chaperones**)
IT 9012-33-3 9041-92-3, α1 Antitrypsin 9054-89-1, Superoxide dismutase
RL: ADV (Adverse effect, including toxicity); BPR (Biological process); BSU (Biological study, unclassified); PRP (Properties); BIOL (Biological study); PROC (Process)
(**mutant**; correction of genetic defects using chemical **chaperones**)

L2 ANSWER 29 OF 33 EMBASE  on STN

AB Recently, we found that different low molecular weight compounds, all known to **stabilize** proteins in their native **conformation**, are effective in correcting the temperature-sensitive protein folding defect associated with the ΔF508 cystic fibrosis transmembrane regulator (CFTR) protein. Here we examined whether the folding of other proteins which exhibit temperature- sensitive folding defects also could be corrected via a similar strategy. Cell lines expressing temperature-sensitive **mutants** of the tumor suppressor protein **p53**, the viral oncogene protein pp60(src), or a ubiquitin activating enzyme E1, were incubated at the nonpermissive temperature (39.5°C) in the presence of glycerol, trimethylamine N-oxide or deuterated water. In each case, the cells exhibited phenotypes similar to those observed when the cells were incubated at the permissive temperature (32.5°C), indicative that the particular protein folding defect had been corrected. These observations, coupled with our earlier work and much older studies in yeast and bacteria, indicate that protein **stabilizing** agents are effective in vivo for correcting protein folding abnormalities. We suggest that this type of approach may prove to be useful for correcting certain protein folding abnormalities associated with human diseases.

ACCESSION NUMBER: 97099314 EMBASE
DOCUMENT NUMBER: 1997099314
TITLE: Correcting temperature-sensitive protein folding defects.
AUTHOR: Brown C.R.; Hong-Brown L.Q.; Welch W.J.
CORPORATE SOURCE: C.R. Brown, UCSF, UC Box 0854, San Francisco, CA 94143, United States. crb@itsa.ucsf.edu
SOURCE: Journal of Clinical Investigation, (1997) 99/6 (1432-1444). Refs: 36

ISSN: 0021-9738 CODEN: JCINAO
COUNTRY: United States
DOCUMENT TYPE: Journal; Article
FILE SEGMENT: 022 Human Genetics
029 Clinical Biochemistry
LANGUAGE: English
SUMMARY LANGUAGE: English

AB Recently, we found that different low molecular weight compounds, all known to **stabilize** proteins in their native **conformation**, are effective in correcting the temperature-sensitive protein folding defect associated with the ΔF508 cystic fibrosis transmembrane regulator (CFTR) protein. Here. . . other proteins which exhibit temperature- sensitive folding defects also could be corrected via a similar strategy. Cell lines expressing temperature-sensitive **mutants** of the tumor suppressor protein **p53**, the viral oncogene protein pp60(src), or a ubiquitin activating enzyme E1, were incubated at the nonpermissive temperature (39.5°C) in the. . . been corrected. These observations, coupled with our earlier work and much older studies in yeast and bacteria, indicate that protein **stabilizing** agents are effective in vivo for correcting protein folding abnormalities. We suggest that this type of approach may prove to. . .

CT Medical Descriptors:
*protein folding
***temperature sensitive mutant**
article
cell growth
cell line
cell structure
gene expression
immunoblotting
priority journal
**chaperone: EC, endogenous compound**
protein kinase p60: EC, endogenous compound
**protein p53: EC, endogenous compound**
ubiquitin protein ligase: EC, endogenous compound

=>

```
FILE 'HCAPLUS' ENTERED AT 22:06:56 ON 24 MAY 2004
USE IS SUBJECT TO THE TERMS OF YOUR STN CUSTOMER AGREEMENT.
PLEASE SEE "HELP USAGETERMS" FOR DETAILS.
COPYRIGHT (C) 2004 AMERICAN CHEMICAL SOCIETY (ACS)

FILE 'BIOSIS' ENTERED AT 22:06:56 ON 24 MAY 2004
COPYRIGHT (C) 2004 BIOLOGICAL ABSTRACTS INC.(R)

FILE 'EMBASE' ENTERED AT 22:06:56 ON 24 MAY 2004
COPYRIGHT (C) 2004 Elsevier Inc. All rights reserved.

FILE 'MEDLINE' ENTERED AT 22:06:56 ON 24 MAY 2004

=> s p53 and (conformation? or stabili?) and chaperon? and (mutant? or
wild(2a)type?)
L1          85 P53 AND (CONFORMATION? OR STABILI?) AND CHAPERON? AND (MUTANT?
               OR WILD(2A) TYPE?)

=> dup rem l1
PROCESSING COMPLETED FOR L1
L2           33 DUP REM L1 (52 DUPLICATES REMOVED)

=> d l2 ibib 1-33
```

(FILE 'HOME' ENTERED AT 22:06:34 ON 24 MAY 2004)

FILE 'HCAPLUS, BIOSIS, EMBASE, MEDLINE' ENTERED AT 22:06:56 ON 24 MAY 2004

L1 85 S P53 AND (CONFORMATION? OR STABILI?) AND CHAPERON? AND (MUTANT

L2 33 DUP REM L1 (52 DUPLICATES REMOVED)

=> s l1 and spectroscop?

L3 3 L1 AND SPECTROSCOP?

=> d l3 abs ibib kwic 1-3

L3 ANSWER 1 OF 3 HCAPLUS COPYRIGHT 2004 ACS on STN

AB We disclose a method of **stabilizing** the native state of a polypeptide, the method comprising exposing the polypeptide to a **stabilizing** mol. capable of binding to the polypeptide at a site which at least partially overlaps a functional site in its native state. The authors describe the isolation and identification of a **stabilizing** peptide CDB3, which is capable of binding the tumor suppressor protein **p53** near its DNA binding site, and **stabilizing** the native form of the protein. Since the binding of DNA itself **stabilizes p53** core domain, and it binds very tightly, **stabilization** by a peptide such as CDB3 is needed only for **mutants** where DNA binding is impaired because **mutant p53** is in denatured **conformation**. Once the protein has bound DNA, the peptide is not needed any more. The ability of CDB3 to induce refolding of **p53** core domain, together with the observation that DNA can displace it from **p53**, led the authors to propose the a "**chaperone**" mechanism for rescuing a denatured oncogenic protein: CDB3 binds only the native state of the oncogenic protein which is able to bind DNA, probably immediately on biosynthesis, and therefore shifts the equilibrium towards the native state. Then DNA can bind the protein, displacing the peptide, which is free again to bind another protein mol. Exemplary design of potential **P53** core domain binding peptides, screening of the CDB peptides for binding **p53** core domain, identification of the **P53** core domain binding peptide CDB3, characterization of CDB3-**P53** core domain binding and binding of fluorescein-labeled CDB3 are reported. **Stabilizing** mols. and/or compns. of the invention can be used in the treatment of any animal or human disease where errors in protein **conformation**, folding and aggregation contribute to the disease. Examples include cancer, cystic fibrosis and neuro-degeneration. In a particularly preferred embodiment, the disease is cancer.

| | |
|---|---|
| ACCESSION NUMBER: | 2003:133296 HCAPLUS |
| DOCUMENT NUMBER: | 138:166255 |
| TITLE: | **Stabilization** of the native **conformation** of a **mutant** tumor suppressor protein **p53** and other **mutant** proteins using CDB3 peptide and other biomolecules and application to treatment of cancer and other diseases |
| INVENTOR(S): | Friedler, Assaf; Fersht, Alan |
| PATENT ASSIGNEE(S): | Medical Research Council, UK |
| SOURCE: | PCT Int. Appl., 73 pp. CODEN: PIXXD2 |
| DOCUMENT TYPE: | Patent |
| LANGUAGE: | English |
| FAMILY ACC. NUM. COUNT: | 1 |
| PATENT INFORMATION: | |

| PATENT NO. | KIND | DATE | APPLICATION NO. | DATE |
|---|---|---|---|---|
| WO 2003014144 | A2 | 20030220 | WO 2002-GB3668 | 20020809 |
| WO 2003014144 | A3 | 20031127 | | |

W: AE, AG, AL, AM, AT, AU, AZ, BA, BB, BG, BR, BY, BZ, CA, CH, CN, CO, CR, CU, CZ, DE, DK, DM, DZ, EC, EE, ES, FI, GB, GD, GE, GH, GM, HR, HU, ID, IL, IN, IS, JP, KE, KG, KP, KR, KZ, LC, LK, LR, LS, LT, LU, LV, MA, MD, MG, MK, MN, MW, MX, MZ, NO, NZ, OM, PH, PL, PT, RO, RU, SD, SE, SG, SI, SK, SL, TJ, TM, TN, TR, TT, TZ, UA, UG, US, UZ, VC, VN, YU, ZA, ZM, ZW, AM, AZ, BY, KG, KZ, MD, RU, TJ, TM

RW: GH, GM, KE, LS, MW, MZ, SD, SL, SZ, TZ, UG, ZM, ZW, AT, BE, BG, CH, CY, CZ, DE, DK, EE, ES, FI, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE, SK, TR, BF, BJ, CF, CG, CI, CM, GA, GN, GQ, GW, ML, MR, NE, SN, TD, TG

| PATENT NO. | KIND | DATE | APPLICATION NO. | DATE |
|---|---|---|---|---|
| EP 1414846 | A2 | 20040506 | EP 2002-749128 | 20020809 |

R: AT, BE, CH, DE, DK, ES, FR, GB, GR, IT, LI, LU, NL, SE, MC, PT, IE, SI, LT, LV, FI, RO, MK, CY, AL, TR, BG, CZ, EE, SK

PRIORITY APPLN. INFO.:

| | | |
|---|---|---|
| GB 2001-19557 | A | 20010810 |
| GB 2001-27917 | A | 20011121 |
| GB 2002-10740 | A | 20020510 |
| WO 2002-GB3668 | W | 20020809 |

TI **Stabilization** of the native **conformation** of a **mutant** tumor suppressor protein **p53** and other **mutant** proteins using CDB3 peptide and other biomolecules and application to treatment of cancer and other diseases

AB We disclose a method of **stabilizing** the native state of a polypeptide, the method comprising exposing the polypeptide to a **stabilizing** mol. capable of binding to the polypeptide at a site which at least partially overlaps a functional site in its native state. The authors describe the isolation and identification of a **stabilizing** peptide CDB3, which is capable of binding the tumor suppressor protein **p53** near its DNA binding site, and **stabilizing** the native form of the protein. Since the binding of DNA itself **stabilizes p53** core domain, and it binds very tightly, **stabilization** by a peptide such as CDB3 is needed only for **mutants** where DNA binding is impaired because **mutant p53** is in denatured **conformation**. Once the protein has bound DNA, the peptide is not needed any more. The ability of CDB3 to induce refolding of **p53** core domain, together with the observation that DNA can displace it from **p53**, led the authors to propose the a "**chaperone**" mechanism for rescuing a denatured oncogenic protein: CDB3 binds only the native state of the oncogenic protein which is able to bind DNA, probably immediately on biosynthesis, and therefore shifts the equilibrium towards the native state. Then DNA can bind the protein, displacing the peptide, which is free again to bind another protein mol. Exemplary design of potential **P53** core domain binding peptides, screening of the CDB peptides for binding **p53** core domain, identification of the **P53** core domain binding peptide CDB3, characterization of CDB3-**P53** core domain binding and binding of fluorescein-labeled CDB3 are reported. **Stabilizing** mols. and/or compns. of the invention can be used in the treatment of any animal or human disease where errors in protein **conformation**, folding and aggregation contribute to the disease. Examples include cancer, cystic fibrosis and neuro-degeneration. In a particularly preferred embodiment, the disease is cancer.

ST tumor suppressor protein **p53** mutation **conformation**
CDB3 peptide anticancer; protein **conformation** mutation

**stabilization** biomol human disease treatment
IT Enzyme functional sites
(active, **stabilizing** biomol. binding to; **stabilization** of native **conformation** of human **mutant** tumor suppressor protein **p53** and other **mutant** proteins using CDB3 peptide and other biomols. and application to treatment of cancer and other diseases)
IT Fluorescence
(anisotropy, biomol. binding detection using; **stabilization** of native **conformation** of human **mutant** tumor suppressor protein **p53** and other **mutant** proteins using CDB3 peptide and other biomols. and application to treatment of diseases)
IT Differential scanning calorimetry
NMR **spectroscopy**
Surface plasmon resonance
(biomol. binding detection using; **stabilization** of native **conformation** of human **mutant** tumor suppressor protein **p53** and other **mutant** proteins using CDB3 peptide and other biomols. and application to treatment of cancer and other diseases)
IT Chromophores
Fluorescent substances
(biomol. derivatized with; **stabilization** of native **conformation** of human **mutant** tumor suppressor protein **p53** and other **mutant** proteins using CDB3 peptide and other biomols. and application to treatment of cancer and other diseases)
IT Amides, biological studies
Amines, biological studies
Phosphates, biological studies
Sulfates, biological studies
Sulfides, biological studies
RL: BUU (Biological use, unclassified); THU (Therapeutic use); BIOL (Biological study); USES (Uses)
(biomol. derivatized with; **stabilization** of native **conformation** of human **mutant** tumor suppressor protein **p53** and other **mutant** proteins using CDB3 peptide and other biomols. and application to treatment of cancer and other diseases)
IT Molecular association
(biomol.-protein native state; **stabilization** of native **conformation** of human **mutant** tumor suppressor protein **p53** and other **mutant** proteins using CDB3 peptide and other biomols. and application to treatment of cancer and other diseases)
IT NMR **spectroscopy**
(heteronuclear double resonance, biomol. binding detection using; **stabilization** of native **conformation** of human **mutant** tumor suppressor protein **p53** and other **mutant** proteins using CDB3 peptide and other biomols. and application to treatment of cancer and other diseases)
IT Protein folding
(induction of refolding; **stabilization** of native **conformation** of human **mutant** tumor suppressor protein **p53** and other **mutant** proteins using CDB3 peptide and other biomols. and application to treatment of cancer and other diseases)
IT Apoptosis

(induction of; **stabilization** of native **conformation**
of human **mutant** tumor suppressor protein **p53** and
other **mutant** proteins using CDB3 peptide and other biomols.
and application to treatment of cancer and other diseases)

IT Carbohydrates, biological studies
DNA
Glycoproteins
Nucleic acids
Oligonucleotides
Peptide nucleic acids
Peptides, biological studies
RNA
RL: BSU (Biological study, unclassified); THU (Therapeutic use); BIOL
(Biological study); USES (Uses)
(**mutant** proteins **stabilization** by;
**stabilization** of native **conformation** of human
**mutant** tumor suppressor protein **p53** and other
**mutant** proteins using CDB3 peptide and other biomols. and
application to treatment of cancer and other diseases)

IT Proteins
RL: BSU (Biological study, unclassified); THU (Therapeutic use); BIOL
(Biological study); USES (Uses)
(oncogenic, **stabilization** of; **stabilization** of
native **conformation** of human **mutant** tumor
suppressor protein **p53** and other **mutant** proteins
using CDB3 peptide and other biomols. and application to treatment of
cancer and other diseases)

IT Dissociation constant
(**p53**-CDB3; **stabilization** of native
**conformation** of human **mutant** tumor suppressor protein
**p53** and other **mutant** proteins using CDB3 peptide and
other biomols. and application to treatment of cancer and other
diseases)

IT Disease, animal
(protein **conformation** error-related; **stabilization**
of native **conformation** of human **mutant** tumor
suppressor protein **p53** and other **mutant** proteins
using CDB3 peptide and other biomols. and application to treatment of
cancer and other diseases)

IT **Conformation**
(protein, **stabilization** of; **stabilization** of native
**conformation** of human **mutant** tumor suppressor protein
**p53** and other **mutant** proteins using CDB3 peptide and
other biomols. and application to treatment of cancer and other
diseases)

IT Denaturation
(protein; **stabilization** of native **conformation** of
human **mutant** tumor suppressor protein **p53** and other
**mutant** proteins using CDB3 peptide and other biomols. and
application to treatment of cancer and other diseases)

IT Antitumor agents
Biochemical molecules
Drug screening
Human
Mutation
Neoplasm
Protein engineering
(**stabilization** of native **conformation** of human
**mutant** tumor suppressor protein **p53** and other

**mutant** proteins using CDB3 peptide and other biomols. and application to treatment of cancer and other diseases)

IT **p53** (protein)
RL: BSU (Biological study, unclassified); THU (Therapeutic use); BIOL (Biological study); USES (Uses)
(**stabilization** of native **conformation** of human **mutant** tumor suppressor protein **p53** and other **mutant** proteins using CDB3 peptide and other biomols. and application to treatment of cancer and other diseases)

IT Proteins
RL: BSU (Biological study, unclassified); BUU (Biological use, unclassified); THU (Therapeutic use); BIOL (Biological study); USES (Uses)
(**stabilization** of; **stabilization** of native **conformation** of human **mutant** tumor suppressor protein **p53** and other **mutant** proteins using CDB3 peptide and other biomols. and application to treatment of cancer and other diseases)

IT Proteins
RL: BSU (Biological study, unclassified); THU (Therapeutic use); BIOL (Biological study); USES (Uses)
(tumor suppressor, **stabilization** of; **stabilization** of native **conformation** of human **mutant** tumor suppressor protein **p53** and other **mutant** proteins using CDB3 peptide and other biomols. and application to treatment of cancer and other diseases)

IT Phenotypes
(**wild-type**, restoring of; **stabilization** of native **conformation** of human **mutant** tumor suppressor protein **p53** and other **mutant** proteins using CDB3 peptide and other biomols. and application to treatment of cancer and other diseases)

IT 497259-83-3P
RL: BPN (Biosynthetic preparation); BSU (Biological study, unclassified); THU (Therapeutic use); BIOL (Biological study); PREP (Preparation); USES (Uses)
(CDB3; **stabilization** of native **conformation** of human **mutant** tumor suppressor protein **p53** and other **mutant** proteins using CDB3 peptide and other biomols. and application to treatment of cancer and other diseases)

IT 56-45-1, L-Serine, biological studies
RL: BSU (Biological study, unclassified); BIOL (Biological study)
(G245S and R249S **p53** mutations; **stabilization** of native **conformation** of human **mutant** tumor suppressor protein **p53** and other **mutant** proteins using CDB3 peptide and other biomols. and application to treatment of cancer and other diseases)

IT 72-19-5, L-Threonine, biological studies
RL: BSU (Biological study, unclassified); BIOL (Biological study)
(I195T **p53** mutation; **stabilization** of native **conformation** of human **mutant** tumor suppressor protein **p53** and other **mutant** proteins using CDB3 peptide and other biomols. and application to treatment of cancer and other diseases)

IT 71-00-1, L-Histidine, biological studies
RL: BSU (Biological study, unclassified); BIOL (Biological study)
(R175H and R273H **p53** mutations; **stabilization** of native **conformation** of human **mutant** tumor suppressor protein **p53** and other **mutant** proteins using CDB3 peptide and other biomols. and application to treatment of

cancer and other diseases)
IT 56-85-9, L-Glutamine, biological studies
RL: BSU (Biological study, unclassified); BIOL (Biological study)
(R248Q **p53** mutation; **stabilization** of native **conformation** of human **mutant** tumor suppressor protein **p53** and other **mutant** proteins using CDB3 peptide and other biomols. and application to treatment of cancer and other diseases)
IT 73-22-3, L-Tryptophan, biological studies
RL: BSU (Biological study, unclassified); BIOL (Biological study)
(R282W **p53** mutation; **stabilization** of native **conformation** of human **mutant** tumor suppressor protein **p53** and other **mutant** proteins using CDB3 peptide and other biomols. and application to treatment of cancer and other diseases)
IT 58-85-5, Biotin 2321-07-5, Fluorescein
RL: BUU (Biological use, unclassified); THU (Therapeutic use); BIOL (Biological study); USES (Uses)
(biomol. derivatized with; **stabilization** of native **conformation** of human **mutant** tumor suppressor protein **p53** and other **mutant** proteins using CDB3 peptide and other biomols. and application to treatment of cancer and other diseases)
IT 497259-83-3DP, fluorescein labeled
RL: BPN (Biosynthetic preparation); BSU (Biological study, unclassified); THU (Therapeutic use); BIOL (Biological study); PREP (Preparation); USES (Uses)
(**stabilization** of native **conformation** of human **mutant** tumor suppressor protein **p53** and other **mutant** proteins using CDB3 peptide and other biomols. and application to treatment of cancer and other diseases)

L3 ANSWER 2 OF 3 EMBASE  on STN
AB **Conformationally** compromised oncogenic **mutants** of the tumor suppressor protein **p53** can, in principle, be rescued by small molecules that bind the native, but not the denatured state. We describe a strategy for the rational search for such molecules. A nine-residue peptide, CDB3, which was derived from a **p53** binding protein, binds to **p53** core domain and **stabilizes** it in vitro. NMR studies showed that CDB3 bound to **p53** at the edge of the DNA binding site, partly overlapping it. The fluorescein-labeled peptide, FL-CDB3, binds **wild-type p53** core domain with a dissociation constant of 0.5 μM, and raises the apparent melting temperatures of **wild-type** and a representative oncogenic **mutant**, R249S core domain, gadd45 DNA competes with CDB3 and displaces it from its binding site. But this competition does not preclude CDB3 from being a lead compound, CDB3 may act as a " **chaperone**" that maintains existing or newly synthesized destabilized **p53 mutants** in a native **conformation** and then allows transfer to specific DNA, which binds more tightly. Indeed, CDB3 restored specific DNA binding activity to a highly destabilized **mutant** 1195T to close to that of **wild-type** level.
ACCESSION NUMBER: 2002045792 EMBASE
TITLE: A peptide that binds and **stabilizes p53** core domain: **Chaperone** strategy for rescue of oncogenic **mutants**.
AUTHOR: Friedler A.; Hansson L.O.; Veprintsev D.B.; Freund S.M.V.;

09/863,976

Rippin T.M.; Nikolova P.V.; Proctor M.R.; Rudiger S.;
Fersht A.R.
CORPORATE SOURCE: A.R. Fersht, Cambridge Univ. Chemical Laboratory, Cambridge Center for Protein Eng., Medical Research Council Centre, Hills Road, Cambridge CB2 2QH, United Kingdom.
arf25@cam.ac.uk
SOURCE: Proceedings of the National Academy of Sciences of the United States of America, (22 Jan 2002) 99/2 (937-942).
Refs: 23
ISSN: 0027-8424 CODEN: PNASA6
COUNTRY: United States
DOCUMENT TYPE: Journal; Article
FILE SEGMENT: 016 Cancer
030 Pharmacology
037 Drug Literature Index
LANGUAGE: English
SUMMARY LANGUAGE: English
TI A peptide that binds and **stabilizes p53** core domain: **Chaperone** strategy for rescue of oncogenic **mutants**.
AB **Conformationally** compromised oncogenic **mutants** of the tumor suppressor protein **p53** can, in principle, be rescued by small molecules that bind the native, but not the denatured state. We describe a strategy for the rational search for such molecules. A nine-residue peptide, CDB3, which was derived from a **p53** binding protein, binds to **p53** core domain and **stabilizes** it in vitro. NMR studies showed that CDB3 bound to **p53** at the edge of the DNA binding site, partly overlapping it. The fluorescein-labeled peptide, FL-CDB3, binds **wild-type p53** core domain with a dissociation constant of 0.5 μM, and raises the apparent melting temperatures of **wild-type** and a representative oncogenic **mutant**, R249S core domain, gadd45 DNA competes with CDB3 and displaces it from its binding site. But this competition does not preclude CDB3 from being a lead compound, CDB3 may act as a "**chaperone**" that maintains existing or newly synthesized destabilized **p53 mutants** in a native **conformation** and then allows transfer to specific DNA, which binds more tightly. Indeed, CDB3 restored specific DNA binding activity to a highly destabilized **mutant** 1195T to close to that of **wild-type** level.
CT Medical Descriptors:
*oncogene
*protein DNA binding
*antineoplastic activity
protein domain
in vitro study
**nuclear magnetic resonance spectroscopy**
dissociation constant
melting point
binding site
**protein conformation**
surface plasmon resonance
fluorescence
anisotropy
differential scanning calorimetry
drug mechanism
human
article
priority journal
***protein p53**

DELACROIX

***chaperone: DV, drug development**
***chaperone: PD, pharmacology**
*protein cdb3: DV, drug development
*protein cdb3: PD, pharmacology
*DNA binding protein
DNA: EC, endogenous compound
fluorescein
synthetic peptide: DV, drug development
synthetic peptide: PD, . . .

L3 ANSWER 3 OF 3 MEDLINE on STN

AB The molecular **chaperone** Hsp90 sequesters oncogenic **mutants** of the tumor suppressor **p53** that have unstable core domains. It is not known whether **p53** is bound in an unfolded, partly folded, or distorted structure, as is unknown for the structure of any bound substrate of Hsp90. It is a particularly difficult problem to analyze in detail the structures of large complexes in which one component is (partly) unfolded. We have shown by transverse relaxation-optimized NMR **spectroscopy** combined with cross-correlated relaxation-enhanced polarization transfer (CRINEPT-TROSY) that **p53** core domain bound in an approximately 200-kDa complex with Hsp90 was predominantly unfolded lacking helical or sheet secondary structure. This mode of binding might be a general feature of substrates of Hsp90.

ACCESSION NUMBER: 2002432245 MEDLINE
DOCUMENT NUMBER: PubMed ID: 12163643
TITLE: CRINEPT-TROSY NMR reveals **p53** core domain bound in an unfolded form to the **chaperone** Hsp90.
AUTHOR: Rudiger Stefan; Freund Stefan M V; Veprintsev Dmitry B; Fersht Alan R
CORPORATE SOURCE: Cambridge Centre for Protein Engineering, Cambridge University and Medical Research Council, MRC Centre, Hills Road, Cambridge CB2 2QH, United Kingdom.
SOURCE: Proceedings of the National Academy of Sciences of the United States of America, (2002 Aug 20) 99 (17) 11085-90. Journal code: 7505876. ISSN: 0027-8424.
PUB. COUNTRY: United States
DOCUMENT TYPE: Journal; Article; (JOURNAL ARTICLE)
LANGUAGE: English
FILE SEGMENT: Priority Journals
ENTRY MONTH: 200209
ENTRY DATE: Entered STN: 20020822
Last Updated on STN: 20030105
Entered Medline: 20020927

TI CRINEPT-TROSY NMR reveals **p53** core domain bound in an unfolded form to the **chaperone** Hsp90.

AB The molecular **chaperone** Hsp90 sequesters oncogenic **mutants** of the tumor suppressor **p53** that have unstable core domains. It is not known whether **p53** is bound in an unfolded, partly folded, or distorted structure, as is unknown for the structure of any bound substrate. . . . detail the structures of large complexes in which one component is (partly) unfolded. We have shown by transverse relaxation-optimized NMR **spectroscopy** combined with cross-correlated relaxation-enhanced polarization transfer (CRINEPT-TROSY) that **p53** core domain bound in an approximately 200-kDa complex with Hsp90 was predominantly unfolded lacking helical or sheet secondary structure. This. . .

CT Check Tags: Support, Non-U.S. Gov't
Binding Sites

*Heat-Shock Proteins 90: CH, chemistry
Heat-Shock Proteins 90: ME, metabolism
**Magnetic Resonance Spectroscopy: MT, methods**
Models, Molecular
**Protein Conformation**
Protein Denaturation
Protein Folding
***Protein p53: CH, chemistry**
**Protein p53: ME, metabolism**
Recombinant Proteins: CH, chemistry
Recombinant Proteins: ME, metabolism
Spectrometry, Fluorescence
Thermodynamics

CN 0 (Heat-Shock Proteins 90); 0 (Protein **p53**); 0 (Recombinant Proteins)

=>

```
     (FILE 'HOME' ENTERED AT 22:06:34 ON 24 MAY 2004)

     FILE 'HCAPLUS, BIOSIS, EMBASE, MEDLINE' ENTERED AT 22:06:56 ON 24 MAY 2004
L1            85 S P53 AND (CONFORMATION? OR STABILI?) AND CHAPERON? AND (MUTANT
L2            33 DUP REM L1 (52 DUPLICATES REMOVED)
L3             3 S L1 AND SPECTROSCOP?
L4           657 S (CONFORMATION? OR STABILI?) AND CHAPERON? AND SPECTROSCOP?
L5           326 DUP REM L4 (331 DUPLICATES REMOVED)
L6            64 S L5 AND (MUTANT? OR WILD(2A)TYPE?)
L7            12 S L6 AND (IDENTIFY? OR ASSAY?)

     FILE 'STNGUIDE' ENTERED AT 22:19:23 ON 24 MAY 2004

     FILE 'HCAPLUS, BIOSIS, EMBASE, MEDLINE' ENTERED AT 22:20:06 ON 24 MAY 2004

=> s l6 and py<=1998
   3 FILES SEARCHED...
L8            14 L6 AND PY<=1998

=> d l8 abs ibib kwic 1-14
```

L8 ANSWER 1 OF 14 HCAPLUS COPYRIGHT 2004 ACS on STN

AB The formation of active membrane-bound nitrate reductase A in Escherichia coli requires the presence of three subunits, NarG, NarH and NarI, as well as fourth protein, NarJ, that is not part of the active nitrate reductase. In narJ strains, both NarG and NarH subunits are associated in an unstable and inactive NarGH complex. A significant activation of this complex was observed in vitro after adding purified NarJ-6His polypeptide to the cell supernatant of a narJ strain. Once the apo-enzyme NarGHI of a narJ **mutant** has become anchored to the membrane via the NarI subunit, it cannot be reactivated by NarJ in vitro. NarJ protein specifically recognizes the catalytic NarG subunit. Fluorescence, ESR (EPR) **spectroscopy** and molybdenum quantification/based on inductively coupled plasma emission **spectroscopy** (ICPES) clearly indicate that, in the absence of NarJ, no molybdenum cofactor is present in the NarGH complex. We propose that NarJ is a specific **chaperone** that binds to NarG and may thus keep it in an appropriate competent-open **conformation** for the molybdenum cofactor insertion to occur, resulting in a catalytically active enzyme. Upon insertion of the molybdenum cofactor into the apo-nitrate reductase, NarJ is then dissociated from the activated enzyme.

| | |
|---|---|
| ACCESSION NUMBER: | 1998:319533 HCAPLUS |
| DOCUMENT NUMBER: | 129:51404 |
| TITLE: | NarJ is a specific **chaperone** required for molybdenum cofactor assembly in nitrate reductase A of Escherichia coli |
| AUTHOR(S): | Blasco, Francis; Dos Santos, Jean-Philippe; Magalon, Axel; Frixon, Chantal; Guigliarelli, Bruno; Santini, Claire-Lise; Giordano, Gerard |
| CORPORATE SOURCE: | Laboratoire de Chimie Bacterienne, IBSM, CNRS, Marseille, 13402, Fr. |
| SOURCE: | Molecular Microbiology (**1998**), 28(3), 435-447<br>CODEN: MOMIEE; ISSN: 0950-382X |
| PUBLISHER: | Blackwell Science Ltd. |
| DOCUMENT TYPE: | Journal |
| LANGUAGE: | English |
| REFERENCE COUNT: | 38 THERE ARE 38 CITED REFERENCES AVAILABLE FOR THIS RECORD. ALL CITATIONS AVAILABLE IN THE RE FORMAT |

TI NarJ is a specific **chaperone** required for molybdenum cofactor assembly in nitrate reductase A of Escherichia coli

SO Molecular Microbiology (**1998**), 28(3), 435-447
CODEN: MOMIEE; ISSN: 0950-382X

AB The formation of active membrane-bound nitrate reductase A in Escherichia coli requires the presence of three subunits, NarG, NarH and NarI, as well as fourth protein, NarJ, that is not part of the active nitrate reductase. In narJ strains, both NarG and NarH subunits are associated in an unstable and inactive NarGH complex. A significant activation of this complex was observed in vitro after adding purified NarJ-6His polypeptide to the cell supernatant of a narJ strain. Once the apo-enzyme NarGHI of a narJ **mutant** has become anchored to the membrane via the NarI subunit, it cannot be reactivated by NarJ in vitro. NarJ protein specifically recognizes the catalytic NarG subunit. Fluorescence, ESR (EPR) **spectroscopy** and molybdenum quantification/based on inductively coupled plasma emission **spectroscopy** (ICPES) clearly indicate that, in the absence of NarJ, no molybdenum cofactor is present in the NarGH complex. We propose that NarJ is a specific **chaperone** that binds to NarG and may thus keep it in an appropriate competent-open **conformation** for the molybdenum cofactor insertion to occur, resulting in a catalytically active enzyme. Upon insertion of the molybdenum cofactor into the apo-nitrate reductase, NarJ is then dissociated from the activated enzyme.

ST NarJ **chaperone** molybdenum cofactor nitrate reductase; Escherichia NarJ molybdenum cofactor nitrate reductase

IT Escherichia coli
(NarJ subunit is a specific **chaperone** that binds to NarG subunit and is required for molybdenum cofactor assembly in nitrate reductase A of Escherichia coli)

IT 9013-03-0, Nitrate reductase
RL: BAC (Biological activity or effector, except adverse); BSU (Biological study, unclassified); PRP (Properties); BIOL (Biological study)
(A; NarJ subunit is a specific **chaperone** that binds to NarG subunit and is required for molybdenum cofactor assembly in nitrate reductase A of Escherichia coli)

IT 73508-07-3, Molybdoenzyme molybdenum cofactor
RL: BPR (Biological process); BSU (Biological study, unclassified); BIOL (Biological study); PROC (Process)
(NarJ subunit is a specific **chaperone** that binds to NarG subunit and is required for molybdenum cofactor assembly in nitrate reductase A of Escherichia coli)

L8 ANSWER 2 OF 14 HCAPLUS 

AB The subunit mol. mass of α-crystallin, like many small heat-shock proteins (sHsps), is around 20 kDa although the protein exists as a large aggregate of average mass around 800 kDa. Despite this large size, a well-resolved 1H NMR spectrum is observed for α-crystallin which arises from short, polar, highly-flexible and solvent-exposed C-terminal extensions in each of the subunits, αA- and αB-crystallin. These extensions are not involved in interactions with other proteins (e.g. β- And γ-crystallins) under non- **chaperone** conditions. As determined by NMR studies on **mutants** of αA-crystallin with alterations in its C-terminal extension, the extensions have an important role in acting as solubilizing agents for the relatively hydrophobic α-crystallin mol. and the high-mol.-weight (HMW) complex that forms during the **chaperone** action. The related sHsp, Hsp25, also exhibits a flexible C-terminal extension. Under **chaperone** conditions, and in the HMW complex isolated from old lenses, the C-terminal extension of the αA-crystallin subunit

maintains its flexibility whereas the αB-crystallin subunit loses, at least partially, its flexibility, implying that it is involved in interaction with the 'substrate' protein. The **conformation** of 'substrate' proteins when they interact with α-crystallin has been probed by 1H NMR **spectroscopy** and it is concluded that α-crystallin interacts with 'substrate' proteins that are in a disordered molten globule state, but only when this state is on its way to large-scale aggregation and precipitation By monitoring the 1H and 31P NMR spectra of α-crystallin in the presence of increasing concns. of urea, it is proposed that α-crystallin adopts a two-domain structure with the larger C-terminal domain unfolding first in the presence of denaturant. All these data have been combined into a model for the quaternary structure of α-crystallin. The model has two layers each of approx. 40 subunits arranged in an annulus or toroid. A large central cavity is present whose entrance is ringed by the flexible C-terminal extensions. A large hydrophobic region in the aggregate is exposed to solution and is available for interaction with 'substrate' proteins during the **chaperone** action.

ACCESSION NUMBER: 1998:264678 HCAPLUS
DOCUMENT NUMBER: 129:64449
TITLE: NMR **spectroscopy** of α-crystallin. Insights into the structure, interactions and **chaperone** action of small heat-shock proteins
AUTHOR(S): Carver, John A.; Lindner, Robyn A.
CORPORATE SOURCE: Department of Chemistry, The University of Wollongong, Wollongong, 2522, Australia
SOURCE: International Journal of Biological Macromolecules ( **1998**), 22(3,4), 197-209
CODEN: IJBMDR; ISSN: 0141-8130
PUBLISHER: Elsevier Science Ltd.
DOCUMENT TYPE: Journal
LANGUAGE: English
REFERENCE COUNT: 53 THERE ARE 53 CITED REFERENCES AVAILABLE FOR THIS RECORD. ALL CITATIONS AVAILABLE IN THE RE FORMAT

TI NMR **spectroscopy** of α-crystallin. Insights into the structure, interactions and **chaperone** action of small heat-shock proteins

SO International Journal of Biological Macromolecules (**1998**), 22(3,4), 197-209
CODEN: IJBMDR; ISSN: 0141-8130

AB The subunit mol. mass of α-crystallin, like many small heat-shock proteins (sHsps), is around 20 kDa although the protein exists as a large aggregate of average mass around 800 kDa. Despite this large size, a well-resolved 1H NMR spectrum is observed for α-crystallin which arises from short, polar, highly-flexible and solvent-exposed C-terminal extensions in each of the subunits, αA- and αB-crystallin. These extensions are not involved in interactions with other proteins (e.g. β- And γ-crystallins) under non- **chaperone** conditions. As determined by NMR studies on **mutants** of αA-crystallin with alterations in its C-terminal extension, the extensions have an important role in acting as solubilizing agents for the relatively hydrophobic α-crystallin mol. and the high-mol.-weight (HMW) complex that forms during the **chaperone** action. The related sHsp, Hsp25, also exhibits a flexible C-terminal extension. Under **chaperone** conditions, and in the HMW complex isolated from old lenses, the C-terminal extension of the αA-crystallin subunit maintains its flexibility whereas the αB-crystallin subunit loses, at least partially, its flexibility, implying that it is involved in interaction with the 'substrate' protein. The **conformation** of

'substrate' proteins when they interact with α-crystallin has been probed by 1H NMR **spectroscopy** and it is concluded that α-crystallin interacts with 'substrate' proteins that are in a disordered molten globule state, but only when this state is on its way to large-scale aggregation and precipitation By monitoring the 1H and 31P NMR spectra of α-crystallin in the presence of increasing concns. of urea, it is proposed that α-crystallin adopts a two-domain structure with the larger C-terminal domain unfolding first in the presence of denaturant. All these data have been combined into a model for the quaternary structure of α-crystallin. The model has two layers each of approx. 40 subunits arranged in an annulus or toroid. A large central cavity is present whose entrance is ringed by the flexible C-terminal extensions. A large hydrophobic region in the aggregate is exposed to solution and is available for interaction with 'substrate' proteins during the **chaperone** action.

ST NMR **spectroscopy** alpha crystallin structure sHSP; **chaperone** heat shock protein interaction crystallin; quaternary structure model alpha crystallin **conformation**

IT Crystal structure
Hydrophobicity
Molecular modeling
NMR (nuclear magnetic resonance)
(NMR **spectroscopy** of α-crystallin: structure, interactions and **chaperone** action of small heat-shock proteins)

IT **Chaperonins**
RL: BPR (Biological process); BSU (Biological study, unclassified); BIOL (Biological study); PROC (Process)
(NMR **spectroscopy** of α-crystallin: structure, interactions and **chaperone** action of small heat-shock proteins)

IT Heat-shock proteins
RL: BPR (Biological process); BSU (Biological study, unclassified); BIOL (Biological study); PROC (Process)
(low-mol.-weight; NMR **spectroscopy** of α-crystallin: structure, interactions and **chaperone** action of small heat-shock proteins)

IT Quaternary structure
(protein, model of; NMR **spectroscopy** of α-crystallin: structure, interactions and **chaperone** action of small heat-shock proteins)

IT **Conformation**
(protein; NMR **spectroscopy** of α-crystallin: structure, interactions and **chaperone** action of small heat-shock proteins)

IT Crystallins
RL: BPR (Biological process); BSU (Biological study, unclassified); PRP (Properties); BIOL (Biological study); PROC (Process)
(α-; NMR **spectroscopy** of α-crystallin: structure, interactions and **chaperone** action of small heat-shock proteins)

IT Crystallins
RL: BPR (Biological process); BSU (Biological study, unclassified); PRP (Properties); BIOL (Biological study); PROC (Process)
(αA-; NMR **spectroscopy** of α-crystallin: structure, interactions and **chaperone** action of small heat-shock proteins)

IT Crystallins
RL: BPR (Biological process); BSU (Biological study, unclassified); PRP

(Properties); BIOL (Biological study); PROC (Process)
(αB-; NMR **spectroscopy** of α-crystallin:
structure, interactions and **chaperone** action of small
heat-shock proteins)

L8 ANSWER 3 OF 14 HCAPLUS COPYRIGHT 2004 ACS on STN

AB Defects in electron transfer flavoprotein (ETF) or its electron acceptor, electron transfer flavoprotein-ubiquinone oxidoreductase (ETF-QO), cause the human inherited metabolic disease glutaric acidemia type II. In this disease, electron transfer from nine primary flavoprotein dehydrogenases to the main respiratory chain is impaired. Among these dehydrogenases are the four chain length-specific flavoprotein dehydrogenases of fatty acid β-oxidation In this investigation, two mutations in the α subunit that have been identified in patients were expressed in Escherichia coli. Of the two **mutant** alleles, αT266M and αG116R, the former is the most frequent mutation found in patients with ETF deficiency. The crystal structure of human ETF shows that αG116 lies in a hydrophobic pocket, under a contact residue of the α/β subunit interface, and that the hydroxyl hydrogen of αT266 is hydrogen-bonded to N(5) of the FAD; the amide backbone hydrogen of αT266 is hydrogen-bonded to C(4)-O of the flavin prosthetic group (Roberts, D. L., Frerman, F. E. and Kim, J-J. P. (1996) Proc. Natl. Acad. Sci. U. S. A. 93, 14355-14360). Stable expression of the αG116R ETF required coexpression of the **chaperonins**, GroEL and GroES. αG116R ETF folds into a **conformation** different from the **wild type**, and is catalytically inactive in crude exts. It is unstable and could not be extensively purified. The αT266M ETF was purified and characterized after **stabilization** to proteolysis in crude exts. Although the global structure of this **mutant** protein is unchanged, its flavin environment is altered as indicated by absorption and CD **spectroscopy** and the kinetics of flavin release from the oxidized and reduced protein. The loss of the hydrogen bond at N(5) of the flavin and the altered flavin binding increase the thermodn. **stability** of the flavin semiquinone by 10-fold relative to the semiquinone of **wild type** ETF. The mutation has relatively little effect on the reductive half-reaction of ETF catalyzed by sarcosine and medium chain acyl-CoA dehydrogenases which reduce the flavin to the semiquinone. However, kcat/Km of ETF-QO in a coupled acyl-CoA:ubiquinone reductase assay with oxidized αT266M ETF as substrate is reduced 33-fold; this decrease is due in largest part to a decrease in the rate of disproportionation of the αT266M ETF semiquinone catalyzed by ETF-QO.

ACCESSION NUMBER: 1997:689123 HCAPLUS
DOCUMENT NUMBER: 128:1285
TITLE: Expression and characterization of two pathogenic mutations in human electron transfer flavoprotein
AUTHOR(S): Salazar, Denise; Zhang, Lening; Degala, Gregory D.; Frerman, Frank E.
CORPORATE SOURCE: Program in Cellular and Developmental Biology and the Department of Pediatrics, University of Colorado School of Medicine, Denver, CO, 80262, USA
SOURCE: Journal of Biological Chemistry (**1997**), 272(42), 26425-26433
CODEN: JBCHA3; ISSN: 0021-9258
PUBLISHER: American Society for Biochemistry and Molecular Biology
DOCUMENT TYPE: Journal
LANGUAGE: English

REFERENCE COUNT: 37 THERE ARE 37 CITED REFERENCES AVAILABLE FOR THIS RECORD. ALL CITATIONS AVAILABLE IN THE RE FORMAT

SO Journal of Biological Chemistry (**1997**), 272(42), 26425-26433
CODEN: JBCHA3; ISSN: 0021-9258

AB Defects in electron transfer flavoprotein (ETF) or its electron acceptor, electron transfer flavoprotein-ubiquinone oxidoreductase (ETF-QO), cause the human inherited metabolic disease glutaric acidemia type II. In this disease, electron transfer from nine primary flavoprotein dehydrogenases to the main respiratory chain is impaired. Among these dehydrogenases are the four chain length-specific flavoprotein dehydrogenases of fatty acid β-oxidation In this investigation, two mutations in the α subunit that have been identified in patients were expressed in Escherichia coli. Of the two **mutant** alleles, αT266M and αG116R, the former is the most frequent mutation found in patients with ETF deficiency. The crystal structure of human ETF shows that αG116 lies in a hydrophobic pocket, under a contact residue of the α/β subunit interface, and that the hydroxyl hydrogen of αT266 is hydrogen-bonded to N(5) of the FAD; the amide backbone hydrogen of αT266 is hydrogen-bonded to C(4)-O of the flavin prosthetic group (Roberts, D. L., Frerman, F. E. and Kim, J-J. P. (1996) Proc. Natl. Acad. Sci. U. S. A. 93, 14355-14360). Stable expression of the αG116R ETF required coexpression of the **chaperonins**, GroEL and GroES. αG116R ETF folds into a **conformation** different from the **wild type**, and is catalytically inactive in crude exts. It is unstable and could not be extensively purified. The αT266M ETF was purified and characterized after **stabilization** to proteolysis in crude exts. Although the global structure of this **mutant** protein is unchanged, its flavin environment is altered as indicated by absorption and CD **spectroscopy** and the kinetics of flavin release from the oxidized and reduced protein. The loss of the hydrogen bond at N(5) of the flavin and the altered flavin binding increase the thermodn. **stability** of the flavin semiquinone by 10-fold relative to the semiquinone of **wild type** ETF. The mutation has relatively little effect on the reductive half-reaction of ETF catalyzed by sarcosine and medium chain acyl-CoA dehydrogenases which reduce the flavin to the semiquinone. However, kcat/Km of ETF-QO in a coupled acyl-CoA:ubiquinone reductase assay with oxidized αT266M ETF as substrate is reduced 33-fold; this decrease is due in largest part to a decrease in the rate of disproportionation of the αT266M ETF semiquinone catalyzed by ETF-QO.

IT **Chaperonins**
RL: BPR (Biological process); BSU (Biological study, unclassified); BIOL (Biological study); PROC (Process)
(GroEL; expression and characterization of two pathogenic mutations in human electron transfer flavoprotein)

IT **Chaperonins**
RL: BPR (Biological process); BSU (Biological study, unclassified); BIOL (Biological study); PROC (Process)
(GroES; expression and characterization of two pathogenic mutations in human electron transfer flavoprotein)

IT **Conformation**
(protein; expression and characterization of two pathogenic mutations in human electron transfer flavoprotein)

L8 ANSWER 4 OF 14 HCAPLUS 

AB α-Crystallins occur as multimeric complexes, which are able to suppress precipitation of unfolding proteins. Although the mechanism of this **chaperone**-like activity is unknown, the affinity of

α-crystallin for aggregation-prone proteins is probably based on hydrophobic interactions. α-Crystallins expose a considerable hydrophobic surface to solution, but nevertheless they are very stable and highly soluble An explanation for this paradox may be that α-crystallin subunits have a polar and unstructured C-terminal extension that functions as a sort of solubilizer. In this paper we have described five αA-crystallins in which charged and hydrophobic residues were inserted in the C-terminal extension. Introduction of lysine, arginine, and aspartate does not substantially influence **chaperone**-like activity. In contrast, introduction of a hydrophobic tryptophan greatly diminishes functional activity. CD expts. indicate that this **mutant** has a normal secondary structure and fluorescence measurements show that the inserted tryptophan is located in polar environment. However, NMR **spectroscopy** clearly demonstrates that the presence of the tryptophan residue dramatically reduces the flexibility of the C-terminal extension. Furthermore, the introduction of this tryptophan results in a considerably decreased thermostability of the protein. We conclude that changing the polarity of the C-terminal extension of αA-crystallin by insertion of a highly hydrophobic residue can seriously disturb structural and functional integrity.

ACCESSION NUMBER: 1996:716584 HCAPLUS
DOCUMENT NUMBER: 126:28206
TITLE: Immobilization of the C-terminal extension of bovine αA-crystallin reduces **chaperone**-like activity
AUTHOR(S): Smulders, Ronald H. P. H.; Carver, John A.; Lindner, Robyn A.; van Boeckel, Martinus A. M.; Bloemendal, Hans; de Jong, Wilfried W.
CORPORATE SOURCE: Dep. Biochem., Univ Nijmegen, Nijmegen, 6500 HB, Neth.
SOURCE: Journal of Biological Chemistry (**1996**), 271(46), 29060-29066
CODEN: JBCHA3; ISSN: 0021-9258
PUBLISHER: American Society for Biochemistry and Molecular Biology
DOCUMENT TYPE: Journal
LANGUAGE: English
TI Immobilization of the C-terminal extension of bovine αA-crystallin reduces **chaperone**-like activity
SO Journal of Biological Chemistry (**1996**), 271(46), 29060-29066
CODEN: JBCHA3; ISSN: 0021-9258
AB α-Crystallins occur as multimeric complexes, which are able to suppress precipitation of unfolding proteins. Although the mechanism of this **chaperone**-like activity is unknown, the affinity of α-crystallin for aggregation-prone proteins is probably based on hydrophobic interactions. α-Crystallins expose a considerable hydrophobic surface to solution, but nevertheless they are very stable and highly soluble An explanation for this paradox may be that α-crystallin subunits have a polar and unstructured C-terminal extension that functions as a sort of solubilizer. In this paper we have described five αA-crystallins in which charged and hydrophobic residues were inserted in the C-terminal extension. Introduction of lysine, arginine, and aspartate does not substantially influence **chaperone**-like activity. In contrast, introduction of a hydrophobic tryptophan greatly diminishes functional activity. CD expts. indicate that this **mutant** has a normal secondary structure and fluorescence measurements show that the inserted tryptophan is located in polar environment. However, NMR **spectroscopy** clearly demonstrates that the presence of the tryptophan residue dramatically

reduces the flexibility of the C-terminal extension. Furthermore, the introduction of this tryptophan results in a considerably decreased thermostability of the protein. We conclude that changing the polarity of the C-terminal extension of αA-crystallin by insertion of a highly hydrophobic residue can seriously disturb structural and functional integrity.

ST crystallin structure **chaperone** function **conformation**

IT **Conformation**
(immobilization of C-terminal extension of bovine αA-crystallin reduces **chaperone**-like activity)

IT **Chaperonins**
RL: BAC (Biological activity or effector, except adverse); BPR (Biological process); BSU (Biological study, unclassified); PEP (Physical, engineering or chemical process); BIOL (Biological study); PROC (Process)
(immobilization of C-terminal extension of bovine αA-crystallin reduces **chaperone**-like activity)

IT Crystallins
RL: BAC (Biological activity or effector, except adverse); BPR (Biological process); BSU (Biological study, unclassified); PEP (Physical, engineering or chemical process); BIOL (Biological study); PROC (Process)
(αA-; immobilization of C-terminal extension of bovine αA-crystallin reduces **chaperone**-like activity)

IT 73-22-3, Tryptophan, biological studies
RL: BPR (Biological process); BSU (Biological study, unclassified); BIOL (Biological study); PROC (Process)
(immobilization of C-terminal extension of bovine αA-crystallin reduces **chaperone**-like activity)

IT 9004-10-8, Insulin, biological studies
RL: BPR (Biological process); BSU (Biological study, unclassified); PEP (Physical, engineering or chemical process); BIOL (Biological study); PROC (Process)
(immobilization of C-terminal extension of bovine αA-crystallin reduces **chaperone**-like activity)

L8 ANSWER 5 OF 14 HCAPLUS COPYRIGHT 2004 ACS on STN

AB α-Crystallin, the major protein of the ocular lens, acts as a mol. **chaperone** by suppressing the nonspecific aggregation of damaged proteins. To investigate the mechanism of the interaction between α-crystallin and substrate proteins, we prepared a tryptophan-free **mutant** of human αA-crystallin and assessed the **conformation** of thermally destabilized proteins captured by this **chaperone** using fluorescence **spectroscopy**. The fluorescence emission characteristics of bound substrates (rhodanese and γ-crystallin) and the results of fluorescence quenching expts. indicate that the proteins captured by α-crystallin are characterized by a very low degree of unfolding. In particular, the structure of rhodanese bound to αA-crystallin appears to be considerably more native-like compared to that of the enzyme bound to the **chaperonin** GroEL. We postulate that α-crystallin (and likely other small heat shock proteins) recognize preferentially the aggregation-prone conformers that occur very early on the denaturation pathway. With its ability to capture and **stabilize** these early non-native structures, α-crystallin appears to be uniquely well suited to **chaperone** the transparency properties of the ocular lens.

ACCESSION NUMBER: 1996:282924 HCAPLUS
DOCUMENT NUMBER: 124:335953
TITLE: **Conformational** properties of substrate proteins bound to a molecular **chaperone**

09/863,976

α-crystallin

AUTHOR(S): Das, Kali P.; Petrash, J. Mark; Surewicz, Witold K.
CORPORATE SOURCE: Mason Eye Inst and Dep. Biochem., Univ. Missouri, Columbia, MO, 65212, USA

SOURCE: Journal of Biological Chemistry (1996), 271(18), 10449-10452
CODEN: JBCHA3; ISSN: 0021-9258
PUBLISHER: American Society for Biochemistry and Molecular Biology
DOCUMENT TYPE: Journal
LANGUAGE: English

TI **Conformational** properties of substrate proteins bound to a molecular **chaperone** α-crystallin

SO Journal of Biological Chemistry (**1996**), 271(18), 10449-10452
CODEN: JBCHA3; ISSN: 0021-9258

AB α-Crystallin, the major protein of the ocular lens, acts as a mol. **chaperone** by suppressing the nonspecific aggregation of damaged proteins. To investigate the mechanism of the interaction between α-crystallin and substrate proteins, we prepared a tryptophan-free **mutant** of human αA-crystallin and assessed the **conformation** of thermally destabilized proteins captured by this **chaperone** using fluorescence **spectroscopy**. The fluorescence emission characteristics of bound substrates (rhodanese and γ-crystallin) and the results of fluorescence quenching expts. indicate that the proteins captured by α-crystallin are characterized by a very low degree of unfolding. In particular, the structure of rhodanese bound to αA-crystallin appears to be considerably more native-like compared to that of the enzyme bound to the **chaperonin** GroEL. We postulate that α-crystallin (and likely other small heat shock proteins) recognize preferentially the aggregation-prone conformers that occur very early on the denaturation pathway. With its ability to capture and **stabilize** these early non-native structures, α-crystallin appears to be uniquely well suited to **chaperone** the transparency properties of the ocular lens.

ST crystallin **chaperone** protein **conformation**

IT **Conformation** and Conformers
(**conformational** properties of substrate proteins bound to mol. **chaperone** α-crystallin)

IT Proteins, biological studies
RL: BPR (Biological process); BSU (Biological study, unclassified); PRP (Properties); BIOL (Biological study); PROC (Process)
(**conformational** properties of substrate proteins bound to mol. **chaperone** α-crystallin)

IT Crystallins
RL: BPR (Biological process); BSU (Biological study, unclassified); BIOL (Biological study); PROC (Process)
(αA-, **conformational** properties of substrate proteins bound to mol. **chaperone** α-crystallin)

IT Crystallins
RL: BPR (Biological process); BSU (Biological study, unclassified); PRP (Properties); BIOL (Biological study); PROC (Process)
(βL-, **conformational** properties of substrate proteins bound to mol. **chaperone** α-crystallin)

IT Crystallins
RL: BPR (Biological process); BSU (Biological study, unclassified); PRP (Properties); BIOL (Biological study); PROC (Process)
(γ-, **conformational** properties of substrate proteins bound to mol. **chaperone** α-crystallin)

IT 9026-04-4, Rhodanese
RL: BPR (Biological process); BSU (Biological study, unclassified); PRP (Properties); BIOL (Biological study); PROC (Process)
(**conformational** properties of substrate proteins bound to mol. **chaperone** α-crystallin)

L8 ANSWER 6 OF 14 HCAPLUS COPYRIGHT 2004 ACS on STN

AB A review, with .apprx.103 refs. The molten globule state of α-lactalbumin is the best-characterized folding intermediate of globular proteins and has been studied intensively by various **spectroscopic** and physicochem. techniques, including stopped-flow CD and fluorescence **spectroscopies**, a hydrogen-exchange technique, 1H-NMR **spectroscopy**, disulfide-exchange chemical, site-directed mutagenesis, and calorimetric techniques. This review summarizes recent studies. Major findings about the structure of the molten globule state are: (1) it is highly heterogeneous, having a highly structured α-helical domain with the β-sheet domain being significantly unfolded; and (2) it is not a nonspecific, collapsed polypeptide but already has a native-like tertiary fold. These structural characteristics are essential to fully understand the thermodn. properties of the molten globule state, which are described in connection with a recently proposed computational approach to predict the structure of the molten globule state of a protein. **Mutant** proteins in which the **stability** of the molten globule state was changed were constructed. Studies of the equilibrium unfolding and kinetic refolding of the **mutant** proteins will provide further insight into the molten globule state as a folding intermediate. In spite of an initial expectation that the structure recognized by an Escherichia coli **chaperone**, GroEL, is the molten globule, the interaction of GroEL with α-lactalbumin in the molten globule state is much weaker than the interaction with more unfolded states of α-lactalbumin, a disulfide-reduced form, and disulfide rearranged species.

ACCESSION NUMBER: 1996:78612 HCAPLUS
DOCUMENT NUMBER: 124:109868
TITLE: The molten globule state of α-lactalbumin
AUTHOR(S): Kuwajima, Kunihiro
CORPORATE SOURCE: Sch. Sci., Univ. Tokyo, Tokyo, 113, Japan
SOURCE: FASEB Journal (**1996**), 10(1), 102-9
CODEN: FAJOEC; ISSN: 0892-6638
PUBLISHER: Federation of American Societies for Experimental Biology
DOCUMENT TYPE: Journal; General Review
LANGUAGE: English

SO FASEB Journal (**1996**), 10(1), 102-9
CODEN: FAJOEC; ISSN: 0892-6638

AB A review, with .apprx.103 refs. The molten globule state of α-lactalbumin is the best-characterized folding intermediate of globular proteins and has been studied intensively by various **spectroscopic** and physicochem. techniques, including stopped-flow CD and fluorescence **spectroscopies**, a hydrogen-exchange technique, 1H-NMR **spectroscopy**, disulfide-exchange chemical, site-directed mutagenesis, and calorimetric techniques. This review summarizes recent studies. Major findings about the structure of the molten globule state are: (1) it is highly heterogeneous, having a highly structured α-helical domain with the β-sheet domain being significantly unfolded; and (2) it is not a nonspecific, collapsed polypeptide but already has a native-like tertiary fold. These structural characteristics are essential to fully understand the thermodn. properties of the molten globule state, which are described in connection with a

recently proposed computational approach to predict the structure of the molten globule state of a protein. **Mutant** proteins in which the **stability** of the molten globule state was changed were constructed. Studies of the equilibrium unfolding and kinetic refolding of the **mutant** proteins will provide further insight into the molten globule state as a folding intermediate. In spite of an initial expectation that the structure recognized by an Escherichia coli **chaperone**, GroEL, is the molten globule, the interaction of GroEL with α-lactalbumin in the molten globule state is much weaker than the interaction with more unfolded states of α-lactalbumin, a disulfide-reduced form, and disulfide rearranged species.

ST review lactalbumin **conformation** molten globule

IT **Conformation** and Conformers
(molten globule; molten globule state of α-lactalbumin)

L8 ANSWER 7 OF 14 HCAPLUS COPYRIGHT 2004 ACS on STN

AB Temperature-sensitive folding (tsf) **mutants** of the phage P22 coat protein prevent newly synthesized polypeptide chains from reaching the **conformation** competent for capsid assembly in cells and can be rescued by the GroEL **chaperone**. Here, the **stabilities** of **wild-type** and 4 tsf **mutant** unpolymd. subunits were investigated. **Wild-type** coat protein subunits denatured at 40°, with a calorimetric enthalpy of .apprx.600 kJ/mol. Comparison with coat protein denaturation within the shell lattice (Tm = 87°, ΔH .apprx. 1700 kJ/mol) indicates that protein-protein interactions within the capsid provide enormous **stabilization**. The melting temps. of the subunits carrying tsf substitutions were similar to **wild-type**. At low temps., the tsf **mutants**, but not the **wild-type**, formed non-covalent dimers, which were dissociated at temps. >30°. **Spectroscopic** and calorimetric studies indicated that the **mutant** proteins have reduced amts. of ordered structure at low temperature, as compared to the **wild-type** protein. Although complex, the in vitro phenotypes are consistent with the in vivo finding that the **mutants** are defective in folding, rather than subunit **stability**. These results suggest a role for incompletely folded subunits as precursors in viral capsid assembly, providing a mechanism of reaching multiple **conformations** in the polymerized form.

ACCESSION NUMBER: 1995:697734 HCAPLUS
DOCUMENT NUMBER: 123:79168
TITLE: **Stability** of **wild-type** and temperature-sensitive protein subunits of the phage P22 capsid
AUTHOR(S): Galisteo, Maria L.; Gordon, Carl L.; King, Jonathan
CORPORATE SOURCE: Fac. Cienc., Univ. Granada, Granada, 18071, Spain
SOURCE: Journal of Biological Chemistry (**1995**), 270(28), 16595-601
CODEN: JBCHA3; ISSN: 0021-9258
PUBLISHER: American Society for Biochemistry and Molecular Bio logy
DOCUMENT TYPE: Journal
LANGUAGE: English

TI **Stability** of **wild-type** and temperature-sensitive protein subunits of the phage P22 capsid

SO Journal of Biological Chemistry (**1995**), 270(28), 16595-601
CODEN: JBCHA3; ISSN: 0021-9258

AB Temperature-sensitive folding (tsf) **mutants** of the phage P22 coat protein prevent newly synthesized polypeptide chains from reaching the **conformation** competent for capsid assembly in cells and can be

rescued by the GroEL **chaperone**. Here, the **stabilities** of **wild-type** and 4 tsf **mutant** unpolymd. subunits were investigated. **Wild-type** coat protein subunits denatured at 40°, with a calorimetric enthalpy of .apprx.600 kJ/mol. Comparison with coat protein denaturation within the shell lattice (Tm = 87°, ΔH .apprx. 1700 kJ/mol) indicates that protein-protein interactions within the capsid provide enormous **stabilization**. The melting temps. of the subunits carrying tsf substitutions were similar to **wild-type**. At low temps., the tsf **mutants**, but not the **wild-type**, formed non-covalent dimers, which were dissociated at temps. >30°. **Spectroscopic** and calorimetric studies indicated that the **mutant** proteins have reduced amts. of ordered structure at low temperature, as compared to the **wild-type** protein. Although complex, the in vitro phenotypes are consistent with the in vivo finding that the **mutants** are defective in folding, rather than subunit **stability**. These results suggest a role for incompletely folded subunits as precursors in viral capsid assembly, providing a mechanism of reaching multiple **conformations** in the polymerized form.

ST protein **stability** phage P22 capsid

IT Virus, bacterial
(P22, **stability** of **wild-type** and temperature-sensitive protein subunits of the phage P22 capsid)

IT Proteins, specific or class
RL: PRP (Properties)
(capsid, **stability** of **wild-type** and temperature-sensitive protein subunits of the phage P22 capsid)

L8 ANSWER 8 OF 14 HCAPLUS COPYRIGHT 2004 ACS on STN

AB Three **mutants** of barnase and a pro-barnase variant, which have a variety of different phys. properties but the same overall protein structure, were analyzed for their folding in the presence of the mol. **chaperone** GroEL. **Mutants** were chosen on the basis that changes in their refolding rate consts. in solution are not correlated with the changes in their **stability**. All barnase variants fold considerably more slowly when bound to GroEL. However, barnase refolding on GroEL parallels that in solution: there is a linear relationship between the refolding rate consts., obtained for **wild-type** and all **mutants** of barnase, in the presence and absence of GroEL. Barnase is synthesized in vivo with a 13 amino acid pro-sequence attached to the N-terminus. The pro-sequence of pro-barnase is shown by NMR **spectroscopy** to be devoid of defined structure. The presence of this pro-sequence has no effect on the overall refolding rate constant or the activity of barnase. In the presence of GroEL, the refolding of pro-barnase is retarded relatively more strongly than that of **wild-type** and the **mutant** barnase proteins, suggesting that the pro-sequence provides addnl. binding sites for the **chaperone**.

ACCESSION NUMBER: 1994:100398 HCAPLUS
DOCUMENT NUMBER: 120:100398
TITLE: Refolding of barnase **mutants** and pro-barnase in the presence and absence of GroEL
AUTHOR(S): Gray, Tamara E.; Eder, Joerg; Bycroft, Mark; Day, Anthony G.; Fersht, Alan R.
CORPORATE SOURCE: Chem. Lab., Univ. Cambridge, Cambridge, CB2 1EW, UK
SOURCE: EMBO Journal (**1993**), 12(11), 4145-50
CODEN: EMJODG; ISSN: 0261-4189
DOCUMENT TYPE: Journal
LANGUAGE: English

TI Refolding of barnase **mutants** and pro-barnase in the presence and absence of GroEL

SO EMBO Journal (**1993**), 12(11), 4145-50
CODEN: EMJODG; ISSN: 0261-4189

AB Three **mutants** of barnase and a pro-barnase variant, which have a variety of different phys. properties but the same overall protein structure, were analyzed for their folding in the presence of the mol. **chaperone** GroEL. **Mutants** were chosen on the basis that changes in their refolding rate consts. in solution are not correlated with the changes in their **stability**. All barnase variants fold considerably more slowly when bound to GroEL. However, barnase refolding on GroEL parallels that in solution: there is a linear relationship between the refolding rate consts., obtained for **wild-type** and all **mutants** of barnase, in the presence and absence of GroEL. Barnase is synthesized in vivo with a 13 amino acid pro-sequence attached to the N-terminus. The pro-sequence of pro-barnase is shown by NMR **spectroscopy** to be devoid of defined structure. The presence of this pro-sequence has no effect on the overall refolding rate constant or the activity of barnase. In the presence of GroEL, the refolding of pro-barnase is retarded relatively more strongly than that of **wild -type** and the **mutant** barnase proteins, suggesting that the pro-sequence provides addnl. binding sites for the **chaperone** .

ST **chaperone** GroEL barnase probarnase refolding; propeptide **chaperone** GroEL interaction barnase refolding

IT **Conformation** and Conformers
(of barnase and probarnase, refolding of, GroEL protein effect on)

IT Proteins, specific or class
RL: BIOL (Biological study)
(**chaperonins** 60, refolding of barnase and probarnase in presence on)

IT **Conformation** and Conformers
(random-coil, of barnase propeptide)

IT 9026-12-4, Barnase
RL: BIOL (Biological study)
(refolding of **mutant** and native forms of, GroEL protein effect on)

L8 ANSWER 9 OF 14 BIOSIS  on STN

AB The expression of the melanin operon (melC) of Streptomyces antibioticus requires the **chaperone**-like protein MelC1 for the incorporation of two copper ions (designated as CuA and CuB) and the secretion of the apotyrosinase (MelC2) via a transient binary complex formation between these two proteins. To investigate whether the copper ligand of tyrosinase is involved in this MelC1cntdotMelC2 binary complex function, six single substitution mutations were introduced into the CuA and CuB sites. These mutations led to differential effects on the **stability**, copper content, and export function of binary complexes but a complete abolishment of tyrosinase activity. The defects in the tyrosinase activity in **mutants** were not because of the impairment of the formation of MelC1cntdotMelC2 complex but rather the failure of MelC2 to be discharged from the copper-activated binary complex. Moreover, the impairments on the discharge of the **mutant** MelC2 from all the **mutant** binary complexes appeared to result from the structural changes in their apoforms or copper-activated forms of the complexes, as evidenced by the fluorescence emission and circular dichroism spectral analysis. Therefore, each of six copper ligands in Streptomyces tyrosinase binuclear copper sites plays a pivotal role in the final maturation and the discharge of tyrosinase from the binary complex

but has a less significant role in its secretion.

ACCESSION NUMBER: 1998:390187 BIOSIS
DOCUMENT NUMBER: PREV199800390187
TITLE: Roles of copper ligands in the activation and secretion of Streptomyces tyrosinase.
AUTHOR(S): Tsai, Tzung-Yuan; Lee, Yan-Hwa Wu [Reprint author]
CORPORATE SOURCE: Inst. Biochemistry, National Yang-Ming Univ., Taipei 112, Taiwan
SOURCE: Journal of Biological Chemistry, (July 24, 1998) Vol. 273, No. 30, pp. 19243-19250. print.
CODEN: JBCHA3. ISSN: 0021-9258.
DOCUMENT TYPE: Article
LANGUAGE: English
ENTRY DATE: Entered STN: 10 Sep 1998
Last Updated on STN: 21 Oct 1998

SO Journal of Biological Chemistry, (July 24, 1998) Vol. 273, No. 30, pp. 19243-19250. print.
CODEN: JBCHA3. ISSN: 0021-9258.

AB The expression of the melanin operon (melC) of Streptomyces antibioticus requires the **chaperone**-like protein MelC1 for the incorporation of two copper ions (designated as CuA and CuB) and the secretion of the apotyrosinase. . . six single substitution mutations were introduced into the CuA and CuB sites. These mutations led to differential effects on the **stability**, copper content, and export function of binary complexes but a complete abolishment of tyrosinase activity. The defects in the tyrosinase activity in **mutants** were not because of the impairment of the formation of MelC1cntdotMelC2 complex but rather the failure of MelC2 to be discharged from the copper-activated binary complex. Moreover, the impairments on the discharge of the **mutant** MelC2 from all the **mutant** binary complexes appeared to result from the structural changes in their apoforms or copper-activated forms of the complexes, as evidenced. . .

IT Methods & Equipment
circular dichroism spectral analysis: analytical method, **spectroscopic** techniques: CT; fluorescence emission **spectroscopy**: analytical method, **spectroscopic** techniques: CB; immunoaffinity chromatography: affinity chromatography, analytical method; immunoblot analysis: Analysis/Characterization Techniques: CB, analytical method; AVIV 60DS spectropolarimeter: AVIV Associates,. . .

L8 ANSWER 10 OF 14 BIOSIS COPYRIGHT 2004 BIOLOGICAL ABSTRACTS INC. on STN

AB DnaJ is a molecular **chaperone**, which not only binds to its various protein substrates, but can also activate the DnaK co-**chaperone** to bind to its various protein substrates as well. DnaJ is a modular protein, which contains a putative zinc finger motif of unknown function. Quantitation of the released Zn(II) ions, upon challenge with p-hydroxymercuriphenylsulfonic acid, and by atomic absorption showed that two Zn(II) ions interact with each monomer of DnaJ. Following the release of Zn(II) ions, the free cysteine residues probably form disulfide bridge(s), which contribute to overcoming the destabilizing effect of losing Zn(II). Supporting this view, infrared and circular dichroism studies show that the DnaJ secondary structure is largely unaffected by the release of Zn(II). Moreover, infrared spectra recorded at different temperatures, as well as scanning calorimetry, show that the Zn(H) ions help to **stabilize** DnaJ's tertiary structure. An internal 57-amino acid deletion of the cysteine-reach region did not noticeably affect the affinity of this **mutant** protein, DnaJ-DELTA-144-200, to bind DnaK nor its ability to stimulate DnaK's

ATPase activity. However, the DnaJ-DELTA-144-200 was unable to induce DnaK to a **conformation** required for the **stabilization** of the DnaK-substrate complex. Additionally, the DnaJ-DELTA-144-200 **mutant** protein alone was unimpaired in its ability to interact with its sigma-32 transcription factor substrate, but exhibited reduced affinity toward its P1 RepA and lambda-P substrates. Finally, these in vitro results correlate well with the in vivo observed partial inhibition of bacteriophage lambda growth in a DnaJ-DELTA-144-200 **mutant** background.

ACCESSION NUMBER: 1996:327422 BIOSIS
DOCUMENT NUMBER: PREV199699049778
TITLE: Structure-function analysis of the zinc finger region of the DnaJ molecular **chaperone**.
AUTHOR(S): Banecki, Bogdan; Liberek, Krzysztof; Wall, Daniel; Wawrzynow, Alicja; Georgopoulos, Costa; Bertoli, Enrico; Tanfani, Fabio; Zylicz, Maciej [Reprint author]
CORPORATE SOURCE: Div. Biophys., Dep. Mol. Biol., Univ. Gdansk, 80-822 Gdansk, Kladki 24, Poland
SOURCE: Journal of Biological Chemistry, (1996) Vol. 271, No. 25, pp. 14840-14848.
CODEN: JBCHA3. ISSN: 0021-9258.
DOCUMENT TYPE: Article
LANGUAGE: English
ENTRY DATE: Entered STN: 26 Jul 1996
Last Updated on STN: 27 Jul 1996

TI Structure-function analysis of the zinc finger region of the DnaJ molecular **chaperone**.

SO Journal of Biological Chemistry, (1996) Vol. 271, No. 25, pp. 14840-14848. CODEN: JBCHA3. ISSN: 0021-9258.

AB DnaJ is a molecular **chaperone**, which not only binds to its various protein substrates, but can also activate the DnaK co-**chaperone** to bind to its various protein substrates as well. DnaJ is a modular protein, which contains a putative zinc finger. . . Zn(II). Moreover, infrared spectra recorded at different temperatures, as well as scanning calorimetry, show that the Zn(H) ions help to **stabilize** DnaJ's tertiary structure. An internal 57-amino acid deletion of the cysteine-reach region did not noticeably affect the affinity of this **mutant** protein, DnaJ-DELTA-144-200, to bind DnaK nor its ability to stimulate DnaK's ATPase activity. However, the DnaJ-DELTA-144-200 was unable to induce DnaK to a **conformation** required for the **stabilization** of the DnaK-substrate complex. Additionally, the DnaJ-DELTA-144-200 **mutant** protein alone was unimpaired in its ability to interact with its sigma-32 transcription factor substrate, but exhibited reduced affinity toward. . . these in vitro results correlate well with the in vivo observed partial inhibition of bacteriophage lambda growth in a DnaJ-DELTA-144-200 **mutant** background.

IT Miscellaneous Descriptors
ANALYTICAL METHOD; BIOCHEMISTRY AND MOLECULAR BIOPHYSICS; CIRCULAR DICHROISM; DNAJ; IR **SPECTROSCOPY**; MOLECULAR **CHAPERONE** ; P-HYDROXYPHENYLMERCURIC ACID; SIGMA FACTOR-32; STRUCTURE-FUNCTION ANALYSIS; TRANSCRIPTION FACTOR; TRANSCRIPTION FACTOR INTERACTION; ZINC FINGER REGION; ZINC ION

L8 ANSWER 11 OF 14 EMBASE COPYRIGHT 2004 ELSEVIER INC. ALL RIGHTS RESERVED. on STN

AB Methanol dehydrogenase (MDH) from Methylobacterium extorquens, Methylophilus methylotrophus. Paracoccus denitrificans and Hyphomicrobium X all contained a single atom of Ca2+ per α2β2 tetramer. The

role of Ca2+ was investigated using the MDH from Methylobacterium extorquens. This was shown to be similar to the MDH from Hyphomicrobium X in having 2 mol of prosthetic group (pyrroloquinoline quinine; PQQ) per mol of tetramer, the PQQ being predominantly in the semiquinone form. MDH isolated from the methanol oxidation **mutants** MoxA-, K- and L- contained no Ca2+ They were identical with the enzyme isolated from **wild-type** bacteria with respect to molecular size, subunit configuration, pl, N-terminal amino acid sequence and **stability** under denaturing conditions (low pH, high urea and high guanidinium chloride) and in the nature and content of the prosthetic group (2 mol of PQQ per mol of MDH). They differed in their lack of Ca2+, the oxidation state of the extracted PQQ (fully oxidized), absence of the semiquinone form of PQQ in the enzyme, reactivity with the suicide inhibitor cyclopropanol and absorption spectrum, which indicated that PQQ is bound differently from that in normal MDH. Incubation of MDH from the **mutants** in calcium salts led to irreversible time-dependent reconstitution of full activity concomitant with restoration of a spectrum corresponding to that of fully reduced normal MDH. It is concluded that Ca2- in MDH is directly or indirectly involved in binding PQQ in the active site. The MoxA-, K- and L- proteins may be involved in maintaining a high Ca2+ concentration in the periplasm. It is more likely, however, that they fill a '**chaperone**' function, **stabilizing** a configuration of MDH which permits incorporation of low concentrations of Ca2+ into the protein.

ACCESSION NUMBER: 92336416 EMBASE
DOCUMENT NUMBER: 1992336416
TITLE: Characterization of **mutant** forms of the quinoprotein methanol dehydrogenase lacking an essential calcium ion.
AUTHOR: Richardson I.W.; Anthony C.
CORPORATE SOURCE: SERC, Centre Molecular Recognition, Department of Biochemistry, University of Southampton,Southampton SO9 3TU, United Kingdom
SOURCE: Biochemical Journal, (1992) 287/3 (709-715).
ISSN: 0264-6021 CODEN: BIJOAK
COUNTRY: United Kingdom
DOCUMENT TYPE: Journal; Article
FILE SEGMENT: 004 Microbiology
029 Clinical Biochemistry
LANGUAGE: English
SUMMARY LANGUAGE: English

TI Characterization of **mutant** forms of the quinoprotein methanol dehydrogenase lacking an essential calcium ion.

SO Biochemical Journal, (1992) 287/3 (709-715).
ISSN: 0264-6021 CODEN: BIJOAK

AB . . . quinine; PQQ) per mol of tetramer, the PQQ being predominantly in the semiquinone form. MDH isolated from the methanol oxidation **mutants** MoxA-, K- and L- contained no Ca2+ They were identical with the enzyme isolated from **wild-type** bacteria with respect to molecular size, subunit configuration, pl, N-terminal amino acid sequence and **stability** under denaturing conditions (low pH, high urea and high guanidinium chloride) and in the nature and content of the prosthetic. . . and absorption spectrum, which indicated that PQQ is bound differently from that in normal MDH. Incubation of MDH from the **mutants** in calcium salts led to irreversible time-dependent reconstitution of full activity concomitant with restoration of a spectrum corresponding to that. . . be involved in maintaining a high Ca2+ concentration in the periplasm. It is more likely, however, that they fill a '**chaperone**' function, **stabilizing** a configuration of

MDH which permits incorporation of low concentrations of Ca2+ into the protein.

CT Medical Descriptors:
***bacterium mutant**
*enzyme structure
**absorption spectroscopy**
amino acid sequence
amino terminal sequence
article
controlled study
enzyme active site
enzyme analysis
enzyme reconstitution
**enzyme stability**
enzyme subunit
methylobacterium
molecular size
nonhuman
oxidation
priority journal
*calcium ion
*oxidoreductase: EC, endogenous compound
enzyme inhibitor
guanidine hydrochloride
methanol
methanol dehydrogenase: EC, endogenous compound
pyrroloquinolinequinone: EC, endogenous compound
urea
unclassified drug

L8 ANSWER 12 OF 14 MEDLINE on STN

AB Chloroplast carbonic anhydrase is dependent on a reducing environment to retain its catalytic activity. To investigate the properties of the three accessible cysteine residues of pea carbonic anhydrase, **mutants** were made in which Ala or Ser substituted for C165, C269, and C272. The **mutants** at position 165 were found to be **spectroscopically** similarly to the **wild-type**. They have a high catalytic activity, and are also sensitive to oxidation. In contrast, both C269 and C272 were found to be critical both for the structure and for the catalytic activity. All **mutants** with substitutions at either of these two positions had to be co-overexpressed with GroES/EL **chaperones** to give soluble enzyme in Escherichia coli. The k(cat) values were decreased by 2 and 3 orders of magnitude for the C272A and C269A **mutants**, respectively, and the Km values were increased approximately 7 times. However, the binding of the inhibitor ethoxyzolamide was only slightly weakened. The near-UV CD spectra were found to be changed in both sign and intensity compared to that of the **wild-type**, and the far-UV spectra indicate some loss of alpha-helix structure. Moreover, the quaternary structure was changed from the **wild-type** octameric to tetrameric in these **mutants**. The results indicate that mutation of either of these cysteines causes minor structural changes around at least one of the two tryptophans of the subunit. Furthermore, the data demonstrate that C269 and C272 are involved in the interaction between subunits and are necessary for a proper structure at the tetramer-tetramer interface.

ACCESSION NUMBER: 97254614 MEDLINE
DOCUMENT NUMBER: PubMed ID: 9100024
TITLE: The sulfhydryl groups of Cys 269 and Cys 272 are critical for the oligomeric state of chloroplast carbonic anhydrase

from Pisum sativum.
AUTHOR: Bjorkbacka H; Johansson I M; Skarfstad E; Forsman C
CORPORATE SOURCE: Department of Biochemistry, Umea University, Sweden.
SOURCE: Biochemistry, **(1997 Apr 8)** 36 (14) 4287-94.
Journal code: 0370623. ISSN: 0006-2960.
PUB. COUNTRY: United States
DOCUMENT TYPE: Journal; Article; (JOURNAL ARTICLE)
LANGUAGE: English
FILE SEGMENT: Priority Journals
ENTRY MONTH: 199705
ENTRY DATE: Entered STN: 19970523
Last Updated on STN: 20000303
Entered Medline: 19970515

SO Biochemistry, **(1997 Apr 8)** 36 (14) 4287-94.
Journal code: 0370623. ISSN: 0006-2960.

AB . . . environment to retain its catalytic activity. To investigate the properties of the three accessible cysteine residues of pea carbonic anhydrase, **mutants** were made in which Ala or Ser substituted for C165, C269, and C272. The **mutants** at position 165 were found to be **spectroscopically** similarly to the **wild-type**. They have a high catalytic activity, and are also sensitive to oxidation. In contrast, both C269 and C272 were found to be critical both for the structure and for the catalytic activity. All **mutants** with substitutions at either of these two positions had to be co-overexpressed with GroES/EL **chaperones** to give soluble enzyme in Escherichia coli. The k(cat) values were decreased by 2 and 3 orders of magnitude for the C272A and C269A **mutants**, respectively, and the Km values were increased approximately 7 times. However, the binding of the inhibitor ethoxyzolamide was only slightly. . . weakened. The near-UV CD spectra were found to be changed in both sign and intensity compared to that of the **wild-type**, and the far-UV spectra indicate some loss of alpha-helix structure. Moreover, the quaternary structure was changed from the **wild-type** octameric to tetrameric in these **mutants**. The results indicate that mutation of either of these cysteines causes minor structural changes around at least one of the. . .

CT . . .
genetics
Ethoxzolamide: ME, metabolism
Ethoxzolamide: PD, pharmacology
Gene Expression
Kinetics
Molecular Weight
Mutagenesis, Site-Directed
*Peas: EN, enzymology
Phosphines: PD, pharmacology
***Protein Conformation**
Protein Structure, Secondary
Protein Structure, Tertiary
Spectrometry, Fluorescence

L8 ANSWER 13 OF 14 MEDLINE on STN

AB One of the major protein components of the ocular lens, alpha-crystallin, is composed of alphaA and alphaB chain subunits that have structural homology to the family of mammalian small heat shock proteins. Like other small heat shock proteins, alpha-crystallin subunits associate to form large oligomeric aggregates that express **chaperone**-like activity, as defined by the ability to suppress nonspecific aggregation of proteins destabilized by treatment with a variety of denaturants including

heat, UV irradiation, and chemical modification. It has been proposed that age-related loss of sequences at the C terminus of the alphaA chain subunit may be a factor in the pathogenesis of cataract due to diminished capacity of the truncated crystallin to protect against nonspecific aggregation of lens proteins. To evaluate the functional consequences of alpha-crystallin modification, two **mutant** forms of alphaA subunits were prepared by site-directed mutagenesis. Like **wild type** (WT), aggregates of approximately 540 kDa were formed from a tryptophan-free alphaA **mutant** (W9F). When added in stoichiometric amounts, both WT and W9F subunits completely suppressed the heat-induced aggregation of aldose reductase. In contrast, subunits encoded by a truncation **mutant** in which the C-terminal 17 residues were deleted (R157STOP), despite having **spectroscopic** properties similar to WT, formed much larger aggregates with a marked reduction in **chaperone**-like activity. Similar results were observed when the **chaperone**-like activity was assessed through inhibition of gamma-crystallin aggregation induced by singlet oxygen. These results demonstrate that the structurally conservative substitution of Phe for Trp-9 has a negligible effect on the functional interaction of alphaA subunits, and that deletion of C-terminal sequences from the alphaA subunit results in substantial loss of **chaperone**-like activity, despite overall preservation of secondary structure.

ACCESSION NUMBER: 97112991 MEDLINE
DOCUMENT NUMBER: PubMed ID: 8943244
TITLE: Cloning, expression, and **chaperone**-like activity of human alphaA-crystallin.
AUTHOR: Andley U P; Mathur S; Griest T A; Petrash J M
CORPORATE SOURCE: Department of Ophthalmology and Visual Sciences, Washington University School of Medicine, St. Louis, Missouri 63110, USA.. petrash@am.seer.wustl.edu
CONTRACT NUMBER: EY05681 (NEI)
EY05856 (NEI)
P30 EY02687 (NEI)
+
SOURCE: Journal of biological chemistry, **(1996 Dec 13)** 271 (50) 31973-80.
Journal code: 2985121R. ISSN: 0021-9258.
PUB. COUNTRY: United States
DOCUMENT TYPE: Journal; Article; (JOURNAL ARTICLE)
LANGUAGE: English
FILE SEGMENT: Priority Journals
OTHER SOURCE: GENBANK-U66582; GENBANK-U66584
ENTRY MONTH: 199701
ENTRY DATE: Entered STN: 19970219
Last Updated on STN: 19980206
Entered Medline: 19970117
TI Cloning, expression, and **chaperone**-like activity of human alphaA-crystallin.
SO Journal of biological chemistry, **(1996 Dec 13)** 271 (50) 31973-80.
Journal code: 2985121R. ISSN: 0021-9258.
AB . . . small heat shock proteins. Like other small heat shock proteins, alpha-crystallin subunits associate to form large oligomeric aggregates that express **chaperone**-like activity, as defined by the ability to suppress nonspecific aggregation of proteins destabilized by treatment with a variety of denaturants. . . . the truncated crystallin to protect against nonspecific aggregation of lens proteins. To evaluate the functional consequences of alpha-crystallin modification, two **mutant** forms of alphaA subunits were prepared by site-directed

mutagenesis. Like **wild type** (WT), aggregates of approximately 540 kDa were formed from a tryptophan-free alphaA **mutant** (W9F). When added in stoichiometric amounts, both WT and W9F subunits completely suppressed the heat-induced aggregation of aldose reductase. In contrast, subunits encoded by a truncation **mutant** in which the C-terminal 17 residues were deleted (R157STOP), despite having **spectroscopic** properties similar to WT, formed much larger aggregates with a marked reduction in **chaperone**-like activity. Similar results were observed when the **chaperone**-like activity was assessed through inhibition of gamma-crystallin aggregation induced by singlet oxygen. These results demonstrate that the structurally conservative substitution. . . functional interaction of alphaA subunits, and that deletion of C-terminal sequences from the alphaA subunit results in substantial loss of **chaperone**-like activity, despite overall preservation of secondary structure.

CT Check Tags: Human; Support, Non-U.S. Gov't; Support, U.S. Gov't, P.H.S.
Aldehyde Reductase: ME, metabolism
Amino Acid Sequence
Base Sequence
**Chaperonins: ME, metabolism**
Circular Dichroism
Cloning, Molecular
Crystallins: CH, chemistry
*Crystallins: GE, genetics
Gene Expression Regulation
Molecular Sequence Data
**Protein Conformation**

CN 0 (**Chaperonins**); 0 (Crystallins); EC 1.1.1.21 (Aldehyde Reductase)

L8 ANSWER 14 OF 14 MEDLINE on STN

AB Although genetic and biochemical evidence has established that GroES is required for the full function of the molecular **chaperone**, GroEL, little is known about the molecular details of their interaction. GroES enhances the cooperativity of ATP binding and hydrolysis by GroEL (refs 4, 5) and is necessary for release and folding of several GroEL substrates. Here we report that native GroES has a highly mobile and accessible polypeptide loop whose mobility and accessibility are lost upon formation of the GroES/GroEL complex. In addition, lesions present in eight independently isolated **mutant** groES alleles map in the mobile loop. Studies with synthetic peptides suggest that the loop binds in a hairpin **conformation** at a site on GroEL that is distinct from the substrate-binding site. Flexibility may be required in the mobile loops on the GroES seven-mer to allow them to bind simultaneously to sites on seven GroEL subunits, which may themselves be able to adopt different arrangements, and thus to modulate allosterically GroEL/substrate affinity.

| | |
|---|---|
| ACCESSION NUMBER: | 93309590 MEDLINE |
| DOCUMENT NUMBER: | PubMed ID: 8100614 |
| TITLE: | Characterization of a functionally important mobile domain of GroES. |
| AUTHOR: | Landry S J; Zeilstra-Ryalls J; Fayet O; Georgopoulos C; Gierasch L M |
| CORPORATE SOURCE: | University of Texas Southwestern Medical Center, Dallas 75235-9041. |
| SOURCE: | Nature, **(1993 Jul 15)** 364 (6434) 255-8. Journal code: 0410462. ISSN: 0028-0836. |
| PUB. COUNTRY: | ENGLAND: United Kingdom |
| DOCUMENT TYPE: | Journal; Article; (JOURNAL ARTICLE) |

LANGUAGE: English
FILE SEGMENT: Priority Journals
ENTRY MONTH: 199308
ENTRY DATE: Entered STN: 19930813
Last Updated on STN: 19950206
Entered Medline: 19930805

SO Nature, **(1993 Jul 15)** 364 (6434) 255-8.
Journal code: 0410462. ISSN: 0028-0836.

AB Although genetic and biochemical evidence has established that GroES is required for the full function of the molecular **chaperone**, GroEL, little is known about the molecular details of their interaction. GroES enhances the cooperativity of ATP binding and hydrolysis. . . whose mobility and accessibility are lost upon formation of the GroES/GroEL complex. In addition, lesions present in eight independently isolated **mutant** groES alleles map in the mobile loop. Studies with synthetic peptides suggest that the loop binds in a hairpin **conformation** at a site on GroEL that is distinct from the substrate-binding site. Flexibility may be required in the mobile loops. . .

CT . . .
DNA, Bacterial
Escherichia coli
GroEL Protein
GroES Protein
*Heat-Shock Proteins: CH, chemistry
Heat-Shock Proteins: GE, genetics
Heat-Shock Proteins: ME, metabolism
**Magnetic Resonance Spectroscopy**
Molecular Sequence Data
Mutation
Peptide Fragments: CS, chemical synthesis
Peptide Fragments: CH, chemistry
Protein Binding
**Protein Conformation**

=>